

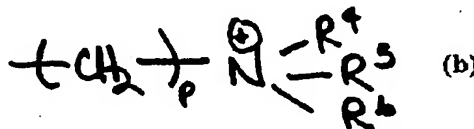
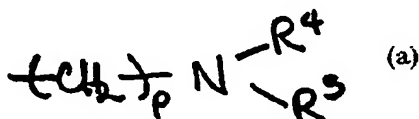
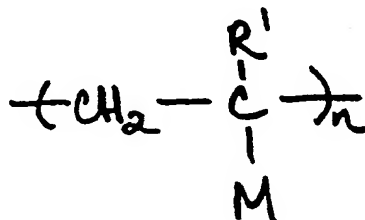
PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>5</sup> : A61K 31/74, 9/00, 9/48, 9/54, 9/52, 9/20, 9/28, 9/14		A1	(11) International Publication Number: <b>WO 94/27620</b>
(21) International Application Number: PCT/US94/06042		(43) International Publication Date: 8 December 1994 (08.12.94)	
(22) International Filing Date: 27 May 1994 (27.05.94)		(81) Designated States: AU, CA, JP, KR, NZ, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(30) Priority Data: 08/071,564 2 June 1993 (02.06.93) US		Published With international search report.	
(71) Applicant: GELTEX, INC. [US/US]; 128 Spring Street, Lexington, MA 02173 (US).			
(72) Inventors: MANDEVILLE, W., Harry, III; 7 Pillings Road, Lynnfield, MA 01940 (US). HOLMES-FARLEY, Stephen, Randall; 20 Norfolk Road, Arlington, MA 02174 (US).			
(74) Agent: WHELAN, Dorothy, P.; Fish & Richardson, 225 Franklin Street, Boston, MA 02110 (US).			

(54) Title: COMPOSITIONS AND PROCESS FOR REMOVING BILE SALTS



## (57) Abstract

A method for removing bile salts from a patient by ion exchange by administering to the patient a therapeutically effective amount of one or more highly crosslinked polymers characterized by a repeat unit having formula (1) or copolymer thereof, where n is an integer; R<sup>1</sup> is H or a C<sub>1</sub>-C<sub>8</sub> alkyl group; M is -C-Z-R<sup>2</sup> or -Z-R<sup>2</sup>; Z is O, NR<sup>3</sup>, S, or (CH<sub>2</sub>)<sub>m</sub>; m = 0-10; R<sup>3</sup> is H or a C<sub>1</sub>-C<sub>8</sub> alkyl group; and R<sup>2</sup> is (a) or (b) where p = 0-10, and each R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, independently, is H, a C<sub>1</sub>-C<sub>8</sub> alkyl group, or an aryl group, the polymers being non-toxic and stable once ingested.

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

- 1 -

## COMPOSITIONS AND PROCESS FOR REMOVING BILE SALTS

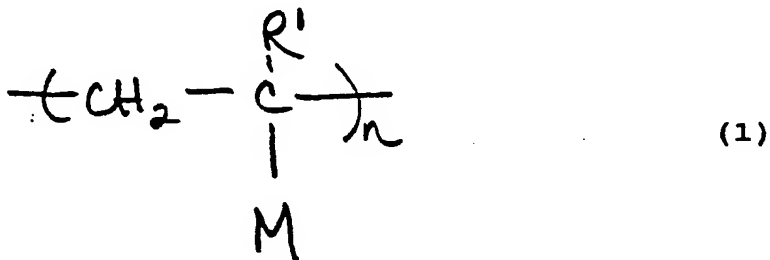
Background of the Invention

5 This invention relates to removing bile salts from a patient.

Sequestering and removing bile salts (e.g., cholate, glycocholate, glycochenocholate, taurocholate, and deoxycholate salts) in a patient can be used to  
 10 reduce the patient's cholesterol level. Ion exchange resins which, when ingested, remove bile salts via the digestive tract, have been used for this purpose. Removal of bile salts will cause the body to prepare more bile salts. Because the biological precursor to bile  
 15 salt is cholesterol, the metabolism of cholesterol to make bile salts is accompanied by a simultaneous reduction in the cholesterol in the patient.

Summary of the Invention

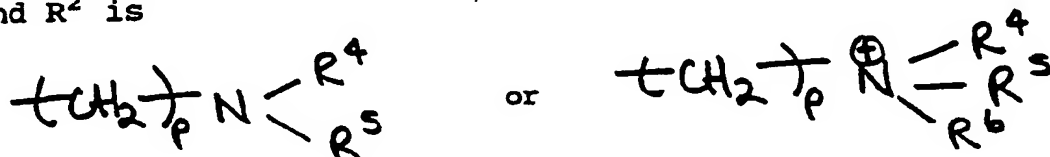
In a first aspect, the invention features a method  
 20 of removing bile salts from a patient by ion exchange that includes administering to the patient a therapeutically effective amount of one or more highly crosslinked polymers that are non-toxic and stable once ingested. The polymers are characterized by a repeat  
 25 unit having the formula



or copolymer thereof, where n is an integer; R<sup>1</sup> is H or a C<sub>1</sub>-C<sub>8</sub> alkyl group (which may be straight chain or branched,

- 2 -

substituted or unsubstituted, e.g., methyl); M is  $-C-Z-R^2$  or  $-Z-R^2$ ; Z is O,  $NR^3$ , S, or  $(CH_2)_m$ ; m = 0-10;  $R^3$  is H or a  $C_1-C_8$  alkyl group (which may be straight chain or  
 5 branched, substituted or unsubstituted, e.g., methyl); and  $R^2$  is



where p = 0-10, and each  $R^4$ ,  $R^5$ , and  $R^6$ , independently, is H, a  $C_1-C_8$  alkyl group (which may be straight chain or  
 10 branched, substituted or unsubstituted, e.g., methyl), or an aryl group (e.g., having one or more rings and which may be substituted or unsubstituted, e.g., phenyl, naphthyl, imidazolyl, or pyridyl).

By "non-toxic" it is meant that when ingested in  
 15 therapeutically effective amounts neither the polymers nor any ions released into the body upon ion exchange are harmful. Preferably, the ions released into the body are actually beneficial to the patient. Such is the case when, for example, the exchangeable ions are natural  
 20 nutrients such as amino acids.

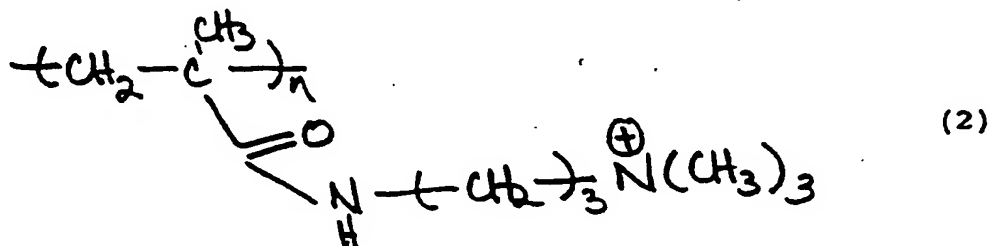
By "stable" it is meant that when ingested in  
 therapeutically effective amounts the polymers do not dissolve or otherwise decompose to form potentially  
 harmful by-products, and remain substantially intact so  
 25 that they can transport ions following ion exchange out of the body.

In preferred embodiments, the polymer is crosslinked by means of a multifunctional crosslinking co-monomer, the co-monomer being present in an amount  
 30 from about 1-25% (more preferably about 2.5-20%) by weight, based upon total monomer weight.

- 3 -

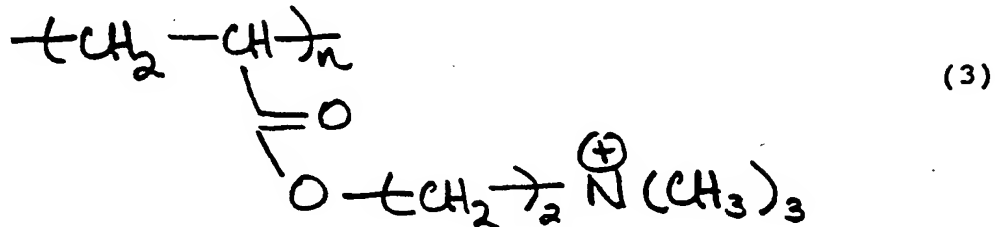
The polymer further preferably includes one or more hydrophobic co-monomers, e.g., styrene, vinyl naphthalene, ethyl vinylbenzene, N-alkyl and N-aryl derivatives of acrylamide and methacrylamide, alkyl and aryl acrylates, alkyl and aryl methacrylates, and fluorinated derivatives of any of these co-monomers (e.g., p-fluorostyrene, pentafluorostyrene, hexafluoroisopropylacrylate, hexafluorobutylmethacrylate, or heptafluorodecylmethacrylate). The alkyl groups are preferably C<sub>1</sub>-C<sub>15</sub> alkyl groups, and may be straight chain, branched, or cyclic (e.g., cyclohexyl), and may further be substituted or unsubstituted. The aryl groups preferably have one or more rings and may be substituted or unsubstituted, e.g., phenyl, naphthyl, imidazolyl, or pyridyl. The polymer may also include one or more positively charged co-monomers, e.g., vinyl pyridine, dimethylaminomethyl styrene, or vinyl imidazole.

One example of a preferred polymer is characterized by a repeat unit having the formula



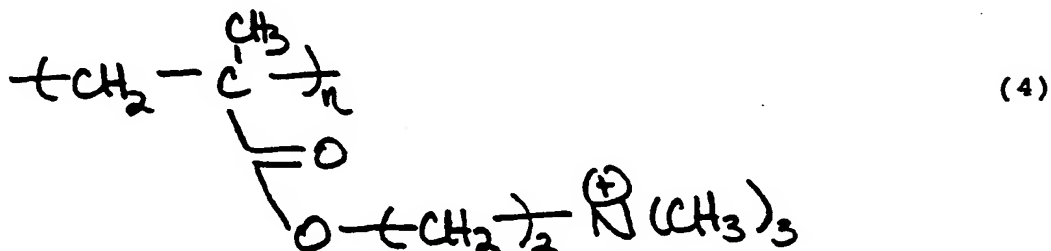
or copolymer thereof. The polymer may further include, as a co-monomer, one or more of the following: n-butylmethacrylamide, hexafluorobutylmethacrylate, heptafluorodecylmethacrylate, styrene or fluorinated derivatives thereof, 2-vinyl naphthalene, 4-vinyl imidazole, vinyl pyridine, trimethylammoniummethylmethacrylate, or trimethylammoniummethylacrylate.

A second example of a preferred polymer is characterized by a repeat unit having the formula



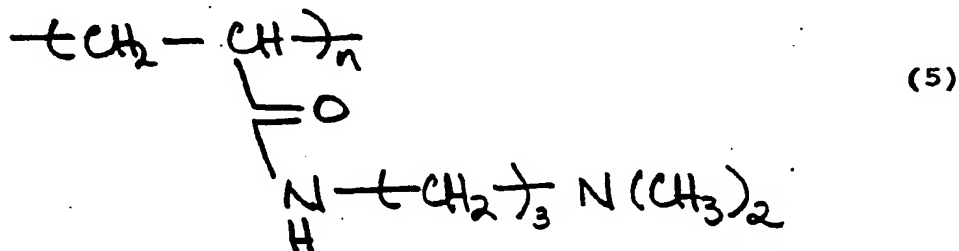
or copolymer thereof. The polymer may also include, as a  
5 co-monomer, one or more of the following:  
isopropylacrylamide, styrene or fluorinated derivatives  
thereof, hexafluoroisopropylacrylate, and  
trimethylammoniummethylemethacrylate.

A third example of a preferred polymer is  
10 characterized by a repeat unit having the formula



or copolymer thereof. The polymer may also include, as a co-monomer, styrene or a fluorinated derivative thereof.

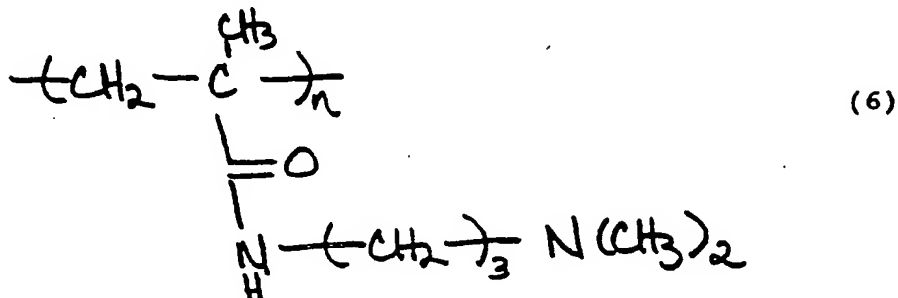
A fourth example of a preferred polymer is  
15 characterized by a repeat unit having the formula



or copolymer thereof.

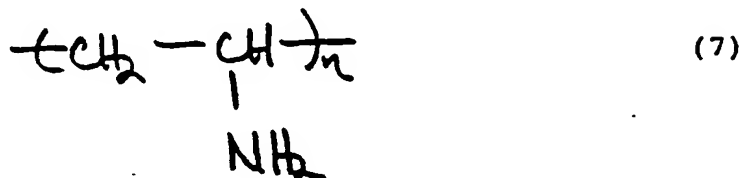
- 5 -

A fifth example of a preferred polymer is characterized by a repeat unit having the formula



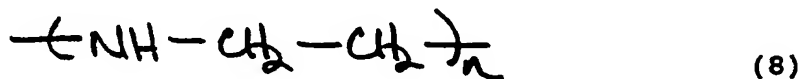
or copolymer thereof.

- 5 A sixth example of a preferred polymer is characterized by a repeat unit having the formula



or copolymer thereof. The polymer may further include, as a co-monomer, ethyl vinylbenzene.

- 10 A seventh example of a preferred polymer is characterized by a repeat unit having the formula



or copolymer thereof.

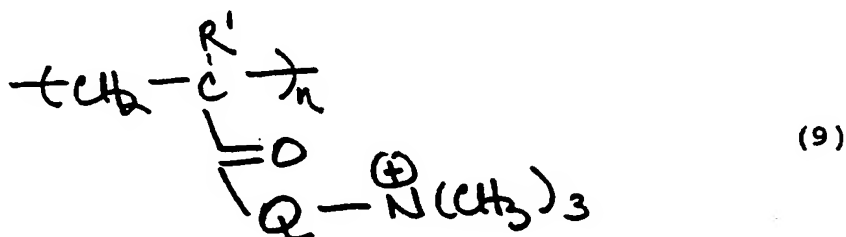
- The polymers may have fixed positive charges, or  
 15 may have the capability of becoming charged upon ingestion at physiological pH. In the latter case, the charged ions also pick up negatively charged counterions upon ingestion that can be exchanged with bile salts. In the case of polymers having fixed positive charges,  
 20 however, the polymer may be provided with one or more

- 6 -

exchangeable counterions. Examples of suitable counterions include  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{CH}_3\text{OSO}_3^-$ ,  $\text{HSO}_4^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{HCO}_3^-$ ,  $\text{CO}_3^-$ , acetate, lactate, succinate, propionate, butyrate, ascorbate, citrate, maleate, folate, an amino acid derivative, a nucleotide, a lipid, or a phospholipid. The counterions may be the same as, or different from, each other. For example, the polymer may contain two different types of counterions, both of which are exchanged for the bile salts being removed. More than one polymer, each having different counterions associated with the fixed charges, may be administered as well.

The invention also features therapeutic compositions for removing bile salts that include a therapeutically effective amount of one or more of the above-described polymers.

In another aspect, the invention features a highly crosslinked polymer composition that includes a polymer characterized by a repeat unit having the formula



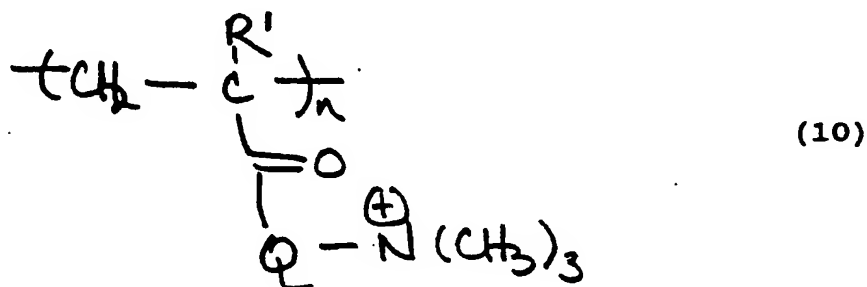
where  $\text{R}^1$  is H or methyl, Q is  $-\text{NH}-(\text{CH}_2)_3-$  or  $-\text{O}-(\text{CH}_2)_2$  and n is an integer, and at least one additional co-monomer selected from the group consisting essentially of vinyl naphthalene, vinylimidazole, fluorinated derivatives of styrene, and fluorinated alkyl methacrylates.

In some preferred embodiments of this aspect,  $\text{R}^1$  is methyl and Q is  $-\text{NH}-(\text{CH}_2)_3-$ . This polymer may further comprise, as a co-monomer, trimethylammoniummethacrylate or trimethylammoniummethacrylate. In other preferred embodiments, Q is  $-\text{O}-(\text{CH}_2)_2$ .

- 7 -

Examples of suitable fluorinated styrene derivatives include p-fluorostyrene and pentafluorostyrene. Examples of suitable fluorinated alkyl methacrylates include hexafluorobutyl methacrylate  
5 and heptadecafluorodecyl methacrylate.

In yet another aspect, the invention features a highly crosslinked polymer composition that includes a polymer characterized by a repeat unit having the formula



- 10 where  $\text{R}^1$  is H or methyl, Q is  $-\text{NH}-(\text{CH}_2)_3-$  or  $-\text{O}-(\text{CH}_2)_2$  and n is an integer, and, as additional co-monomers, (a) styrene and (b) trimethylammoniummethylacrylate or trimethylammoniummethylethylmethacrylate when  $\text{R}^1$  is methyl and Q is  $-\text{NH}-(\text{CH}_2)_3-$ , and  
15 methylacrylamidopropyltrimethylammonium when  $\text{R}^1$  is H or methyl and Q is  $-\text{O}-(\text{CH}_2)_2$ .

In an additional aspect, the invention features a method of synthesizing a highly crosslinked polymer having hydrophilic and hydrophobic units that includes  
20 reacting hydrophilic and hydrophobic monomers in the presence of an alcoholic solvent.

The invention provides an effective treatment for removing bile salts from a patient (and thereby reducing the patient's cholesterol level). The compositions are  
25 non-toxic and stable when ingested in therapeutically effective amounts. They are also tasteless (in the absence of added flavoring) and odorless, as well as being non-constipating and non-gritty (when measured

- 8 -

relative to gels such as cholestyramine) such that irritation to the gastrointestinal tract upon ingestion is minimized.

The invention further provides an effective  
5 synthesis for polymers having hydrophilic and hydrophobic units by conducting the reaction in the presence of an alcoholic solvent not normally considered a good polymerization solvent due to its chain transfer properties.

10 Other features and advantages will be apparent from the following description of the preferred embodiments thereof and from the claims.

Description of the Preferred Embodiments  
Compositions

15 Preferred polymers have the formulae set forth in the Summary of the Invention, above. The polymers are highly crosslinked. The high level of crosslinking makes the polymers completely insoluble and thus limits their activity to the gastrointestinal tract  
20 only. Thus, the polymers are non-systemic in their activity and will lead to reduced side-effects in the patient.

The polymers are preferably crosslinked by adding a crosslinking co-monomer to the reaction mixture  
25 during polymerization. Examples of suitable crosslinking co-monomers are diacrylates and dimethacrylates (e.g., ethylene glycol diacrylate, propylene glycol diacrylate, butylene glycol diacrylate, ethylene glycol dimethacrylate, propylene glycol dimethacrylate, butylene  
30 glycol dimethacrylate, polyethyleneglycol dimethacrylate, polyethyleneglycol diacrylate), methylene bisacrylamide, methylene bismethacrylamide, ethylene bisacrylamide, ethylenebismethacrylamide, ethylidene bisacrylamide, divinyl benzene, bisphenol A dimethacrylate, and  
35 bisphenol A diacrylate. These crosslinking monomers are

- 9 -

either commercially available or are prepared as described in Mandeville et al., "Process for Adjusting Ion Concentration in a Patient and Compositions Therefor," U.S.S.N. 08/065,113, filed May 20, 1993,

5 assigned to the same assignee as the present application and hereby incorporated by reference. The amount of crosslinking co-monomer is typically between 1.0 and 25 weight %, based upon combined weight of crosslinking co-monomer and monomer, with 2.5-20% being preferred.

10 Preferably, the polymer includes one or more co-monomers that increase the overall hydrophobicity of the polymer. Because bile salts are hydrophobic, the hydrophobic co-monomer aids in maximizing the selectivity of the interaction of the polymer with the bile salts.

15 Examples of suitable hydrophobic co-monomers include, e.g., acrylamide, methacrylamide, and N-alkyl (e.g., methyl, ethyl, isopropyl, butyl, hexyl, dodecyl, cyclohexyl, dicyclohexyl) and N-aryl (e.g., phenyl, diphenyl) derivatives thereof; alkyl and aryl acrylates  
20 and methacrylates (e.g., ethyl, propyl, butyl, dodecyl), and fluorinated derivatives thereof (e.g., hexafluoroisopropyl acrylate, hexafluorobutyl methacrylate, heptafluorodecyl acrylate); styrene and derivatives thereof (e.g., dimethylaminomethyl  
25 styrene and fluorinated derivatives, e.g., p-fluorostyrene, pentafluorostyrene); ethylvinylbenzene; vinyl naphthalene; vinyl pyridine; and vinyl imidazole. The amount of hydrophobic co-monomer used in the preparation of these polymers is from 1 to 75% by weight,  
30 preferably from 3 to 65%.

The level of hydrophobicity needed may also be achieved simply by appropriate choice of crosslinking co-monomer. For example, divinylbenzene is a suitable crosslinking co-monomer and is hydrophobic as well. In  
35 addition, the main "impurity" in divinylbenzene is

- 10 -

ethylvinylbenzene, a hydrophobic, polymerizable monomer which will also contribute to the overall hydrophobicity of the polymer. Other hydrophobic crosslinking co-monomers include bisphenol A diacrylate and bisphenol A dimethacrylate.

#### Examples

##### A. Polymer Preparation

1. Preparation of Poly  
(methacrylamidopropyltrimethylammonium  
chloride) (PolyMAPTAC)

To a 1000 mL, three-necked flask, round-bottomed flask was added the following:  
methacrylamidopropyltrimethylammonium chloride (MAPTAC) (40 mL of a 50% aqueous solution, 21 g), ethylene glycol dimethacrylate crosslinking co-monomer (5.00 g, 4.76 mL), ethyl acetate (200 mL), and 2-propanol (200 mL). The resulting solution was clear. Next, the polymerization initiator AIBN (0.1 g) was added and the reaction mixture was heated to 65°C. When the temperature reached 65°C, the solution was degassed with nitrogen for 5 minutes, at which point it turned cloudy, indicating that polymerization was proceeding. The reaction was maintained at 65°C for another 3 hours and then allowed to cool to room temperature.

The resulting polymer (which was hard and sticky) was combined with 500 mL of water to soften it, and then transferred to a blender where it was blended with 1500 mL of 2-propanol and centrifuged. The mixture was then decanted and transferred to another blender with the aid of 100 mL of water. 800 mL of 2-propanol was then added and the mixture was blended, allowed to settle, and decanted. The mixture was then combined with 1000 mL of 2-propanol, blended, filtered, and vacuum-dried to afford 12.6 g of polymer.

PolyMAPTAC crosslinked with 0.5% methylenebismethacrylamide crosslinking co-monomer;

- 11 -

polyMAPTAC crosslinked with 10% methylenebismethacrylamide crosslinking co-monomer; and polyMAPTAC crosslinked with 10% divinylbenzene crosslinking co-monomer were prepared in analogous fashion.

## 2. Preparation of Poly (vinylamine)

The first step involved the preparation of ethylidenebisacetamide. Acetamide (118 g), acetaldehyde (44.06 g), copper acetate (0.2 g), and water (300 mL) were placed in a 1 L three neck flask fitted with condenser, thermometer, and mechanical stirrer. Concentrated HCl (34 mL) was added and the mixture was heated to 45-50°C with stirring for 24 h. The water was then removed in vacuo to leave a thick sludge which formed crystals on cooling to 5°C. Acetone (200 mL) was added and stirred for a few minutes, after which the solid was filtered off and discarded. The acetone was cooled to 0°C and solid was filtered off. This solid was rinsed in 500 mL acetone and air dried 18 h to yield 31.5 g of ethylidenebisacetamide.

The next step involved the preparation of vinylacetamide from ethylidenebisacetamide. Ethylidenebisacetamide (31.05 g), calcium carbonate (2 g) and celite 541 (2 g) were placed in a 500 mL three neck flask fitted with a thermometer, a mechanical stirrer, and a distilling head atop a Vigroux column. The mixture was vacuum distilled at 35 mm Hg by heating the pot to 180-225°C. Only a single fraction was collected (10.8 g) which contained a large portion of acetamide in addition to the product (determined by NMR). This solid product was dissolved in isopropanol (30 mL) to form the crude vinylacetamide solution used for polymerization.

Crude vinylacetamide solution (15 mL), divinylbenzene (1 g, technical grade, 55% pure, mixed isomers), and AIBN (0.3 g) were mixed and heated to

- 12 -

reflux under a nitrogen atmosphere for 90 min, forming a solid precipitate. The solution was cooled, isopropanol (50 mL) was added, and the solid was collected by centrifugation. The solid was rinsed twice in

5 isopropanol, once in water, and dried in a vacuum oven to yield 0.8 g of poly(vinylacetamide), which was used to prepare poly(vinylamine as follows).

Poly(vinylacetamide) (0.79 g) was placed in a 100 mL one neck flask containing water (25 mL) and conc. HCl  
10 (25 mL). The mixture was refluxed for 5 days, after which the solid was filtered off, rinsed once in water, twice in isopropanol, and dried in a vacuum oven to yield 0.77 g of product. Infrared spectroscopy indicated that a significant amount of the amide ( $1656\text{ cm}^{-1}$ ) remained and  
15 that not much amine ( $1606\text{ cm}^{-1}$ ) was formed. The product of this reaction (~0.84 g) was suspended in NaOH (46 g) and water (46 g) and heated to boiling (~ $140^{\circ}\text{C}$ ). Due to foaming the temperature was reduced and maintained at ~ $100^{\circ}\text{C}$  for 2 h. Water (100 mL) was added and the solid  
20 collected by filtration. After rinsing once in water the solid was suspended in water (500 mL) and adjusted to pH 5 with acetic acid. The solid was again filtered off, rinsed with water, then isopropanol, and dried in a vacuum oven to yield 0.51 g of product. Infrared  
25 spectroscopy indicated that significant amine had been formed.

### 3. Preparation of Poly(3-dimethylaminopropylacrylamide) (DMAPA)

Dimethylaminopropylacrylamide (10 g) and  
30 methylenebisacrylamide crosslinking co-monomer (1.1 g) were dissolved in 50 mL of water in a 100 mL three neck flask. The solution was stirred under nitrogen for 10 minutes. Potassium persulfate (0.3 g) and sodium metabisulfite (0.3 g) were each dissolved in 2-3 mL of  
35 water and then mixed. After a few seconds this solution

- 13 -

was added to the monomer solution, still under nitrogen. A gel formed immediately and was allowed to sit overnight. The gel was removed and blended with 500 mL of isopropanol. The solid was filtered off and rinsed  
5 three times with acetone. The solid white powder was filtered off and dried in a vacuum oven to yield 6.1 g.

4. Preparation of  
Poly(dimethylaminopropylacrylamide  
hydrochloride) (DMAPA·HCl)

10 Dimethylaminopropylacrylamide (20.10 g) was dissolved in water (100 mL) and neutralized with concentrated HCl to pH 6.95. Methylenebisacrylamide crosslinking co-monomer (2.2 g) and water (100 mL) were added and warmed (34°C) to dissolve. Potassium  
15 persulfate (0.2 g) and potassium metabisulfite (0.2 g) were added with stirring. After gellation, the solution was allowed to sit for 6 h, blended with isopropanol (600 mL) three times, and dried in a vacuum oven to yield 14.47 g of the title polymer.

20 PolyDMPA·HCl crosslinked with 10% methylenebismethacrylamide crosslinking co-monomer was prepared in analogous fashion.

25 5. Preparation of  
Poly(dimethylaminopropylmethacrylamide  
hydrochloride) (DMPMA·HCl)

Dimethylaminopropylmethacrylamide (20.0 g) was dissolved in water (100 mL) and neutralized with concentrated HCl to pH 6.94. Methylenebisacrylamide crosslinking co-monomer (2.2 g) was added and the  
30 solution was warmed (39°C) to dissolve. Potassium persulfate (0.3 g) and potassium metabisulfite (0.3 g) were added with stirring under a nitrogen atmosphere. After gellation, the solution was allowed to sit overnight, blended with isopropanol (500 mL) twice, and  
35 dried in a vacuum oven to yield 27.65 g of product. Some of the solid (3.2 g; sieved to

- 14 -

-80/+200 mesh size) was stirred in water (100 mL) for 50 min, additional water (100 mL) was added and the solution stirred for 36 min. The solid was collected by centrifugation, resuspended in water (400 mL), stirred

5 150 min, and again collected by centrifugation. The solid was finally resuspended in water (500 mL), stirred 90 min, and collected by filtration. The solid was dried in a vacuum oven to yield 0.28 g of the title polymer.

10 6. Preparation of  
Poly(methacrylamidopropyltrimethylammonium  
chloride) co-poly(n-butylmethacrylamide)  
(MAPTAC co-BuMA)

The co-monomer n-butylmethacrylamide (BuMA) was prepared as follows.

15 Methacryloyl chloride (48.4mL, 52.3g, 0.500mol) was dissolved in tetrahydrofuran (300 mL) in a 1 L flask and placed in an ice bath. A solution containing butylamine (36.6 g) and triethylamine (55.6 g) was added dropwise, maintaining the temperature at 5-15°C. After

20 addition the solution was stirred for 5 min and the solid triethylamine hydrochloride was filtered off and discarded. The solvent was removed in vacuo from the mother liquor and the resulting yellow oil was used without further purification. The yield was 71.58g of

25 BuMA co-monomer.

To a 1000 mL, three-necked flask, round-bottomed flask was added the following:  
methacrylamidopropyltrimethylammonium chloride (MAPTAC) (108 mL of a 50% aqueous solution, 56.8 g), ethylene

30 glycol dimethacrylate crosslinking co-monomer (19.62 g), BuMA co-monomer (12.12g), and 2-propanol (850 mL). The resulting solution was clear. Next, the reaction mixture was heated to 40°C while being degassed with nitrogen. When the solution had reached 40°C, the catalyst,

35 consisting of a solution of potassium persulfate (0.75g) and potassium metabisulfate (0.75g) in 25mL of water was

- 15 -

added. The solution immediately began to turn cloudy, indicating that polymerization was proceeding. The reaction was maintained at 40°C for 24 hours and then allowed to cool to room temperature.

5 The resulting polymer was filtered and washed on the funnel with isopropanol and vacuum dried to afford 64.54 g of the title polymer.

Polymer for testing was washed two times with 800mL of water each time, followed by two washes with 500  
10 mL of methanol each time to give 34.5 g of purified polymer.

A crosslinked MAPTAC co-BuMA copolymer was also prepared using propylene glycol dimethacrylate, rather than ethylene glycol dimethacrylate, as the crosslinking  
15 co-monomer, as follows.

To a 1000 mL, three-necked flask, round-bottomed flask was added the following:  
methacrylamidopropyltrimethylammonium chloride (MAPTAC) (60 mL of a 50% aqueous solution, 31.5 g), propylene  
20 glycol dimethacrylate crosslinking co-monomer (9.81 g), BuMA co-monomer (6.06g), and 2-propanol (300 mL). The resulting solution was clear. Next, the reaction mixture was heated to 70°C while being degassed with nitrogen. When the solution had reached 70°C, the catalyst, AIBN  
25 (0.50g), was added. The solution immediately began to turn cloudy, indicating that polymerization was proceeding. The reaction was maintained at 70°C for 6 hours and then allowed to cool to room temperature.

The resulting polymer was filtered and washed on  
30 the funnel with isopropanol and vacuum dried to afford 23.3 g of polymer.

MAPTAC coBuMA (5%) crosslinked with 24% ethyleneglycoldimethacrylate crosslinking co-monomer, MAPTAC coBuMA (2%) crosslinked with 0.5%  
35 methylenebismethacrylamide crosslinking co-monomer, and

- 16 -

MAPTAC coBuMA (14%) crosslinked with 22% propyleneglycoldimethacrylate crosslinking co-monomer were prepared in analogous fashion by adjusting the ratios of starting monomers.

5           7.     Preparation of  
              Poly(methacrylamidopropyltrimethylammonium  
              chloride) co-poly(styrene) (MAPTAC co-Sty)

To a 1000 mL, three-necked flask, round-bottomed flask was added the following:

10 methacrylamidopropyltrimethyl-ammonium chloride (MAPTAC)  
(60 mL of a 50% aqueous solution, 31.5 g), divinyl  
benzene crosslinking co-monomer (2.00 g), styrene co-  
monomer (1.75g), and 2-propanol (300 mL). The resulting  
solution was clear. Next, the reaction mixture was  
15 heated to 60°C while being degassed with nitrogen. When  
the solution had reached 60°C, the catalyst, AIBN  
(0.50g), was added. The solution immediately began to  
turn cloudy, indicating that polymerization was  
proceeding. The reaction was maintained at 60°C for 24  
20 hours and then allowed to cool to room temperature.  
After about 7 hours the mixture had become very thick and  
100 mL additional isopropanol was added to allow for  
better stirring.

The resulting polymer was filtered and washed on  
25 the funnel with isopropanol and vacuum dried to afford  
30.9 g of the title polymer.

Polymer for testing was washed two times with 1000  
mL of water each time followed by two washes with 800 mL  
of methanol each time to give 28.0 g of purified polymer.

30           MAPTAC co-Sty (13%) crosslinked with 7.5%  
butyleneglycoldimethacrylate crosslinking co-monomer,  
MAPTAC co-Sty (13%) crosslinked with 20%  
butyleneglycoldimethacrylate crosslinking co-monomer,  
MAPTAC co-Sty (19%) crosslinked with 6% divinylbenzene  
35 co-monomer, MAPTAC co-Sty (23%) crosslinked with 7%  
divinylbenzene co-monomer, MAPTAC co-Sty (30%)

- 17 -

crosslinked with 6% divinylbenzene co-monomer, and MAPTAC co-Sty (38%) crosslinked with 6% divinylbenzene co-monomer were prepared in analogous fashion by varying the ratios of starting monomers.

- 5           8.    Preparation of  
              Poly(methacrylamidopropyltrimethylammonium  
              chloride) co-poly(vinyl naphthalene) (MAPTAC  
              co-VN)
- 

              To a 1000 mL, three-necked, round-bottomed flask  
10 was added the following:  
              methacrylamidopropyltrimethylammonium chloride (MAPTAC)  
              (40 mL of a 50% aqueous solution, 21.0 g), divinyl  
              benzene crosslinking co-monomer (2.25 g), 2-  
              vinyl naphthalene co-monomer (10.5 g), and 2-propanol (320  
15 mL). The resulting solution was clear. Next, the  
              reaction mixture was heated to 65°C while being degassed  
              with nitrogen. When the solution had reached 65°C, the  
              catalyst, AIBN (0.50g), was added. The solution  
              immediately began to turn cloudy, indicating that  
20 polymerization was proceeding. The reaction was  
              maintained at 65°C for 20 hours and then allowed to cool  
              to room temperature.

              The resulting polymer was filtered and washed on  
              the funnel with isopropanol and then immediately slurried  
25 in 400 mL of distilled water. The mixture was stirred  
              for 1/2 hour and then filtered. The water wash was  
              repeated one more time. The filter cake was then  
              slurried in 400 mL of methanol and stirred for 1/2 hour.  
              The mixture was filtered and the methanol slurry was  
30 repeated one more time. Vacuum drying afforded 22.11 g,  
              65.5% of the title polymer.

              MAPTAC co-VN (39%) crosslinked with 5% divinyl  
              benzene crosslinking co-monomer was prepared in analogous  
              fashion by varying the ratio of starting monomers.

- 18 -

9. Preparation of  
Poly(methacrylamidopropyltrimethylammonium  
chloride) co-poly(1-vinyl imidazole) (MAPTAC  
co-VI)

---

5 To a 1000 mL, three-necked, round-bottomed flask  
was added the following:  
methacrylamidopropyltrimethylammonium chloride (MAPTAC)  
(40 mL of a 50% aqueous solution, 21.0 g), divinyl  
benzene crosslinking co-monomer (2.25 g), 1-  
10 vinylimidazole co-monomer (12.54 g), and 2-propanol (300  
mL). The resulting solution was clear. Next, the  
reaction mixture was heated to 65°C while being degassed  
with nitrogen. When the solution had reached 65°C, the  
catalyst, AIBN (0.50g), was added. The solution  
15 immediately began to turn cloudy, indicating that  
polymerization was proceeding. The reaction was  
maintained at 65°C for 20 hours and then allowed to cool  
to room temperature.

The resulting polymer was filtered and washed on  
20 the funnel with isopropanol, and then immediately  
slurried in 500 mL of distilled water. The mixture was  
stirred for 1/2 hour and then filtered. The water wash  
was repeated one more time. The filter cake was then  
slurried in 400 mL of methanol and stirred for 1/2 hour.  
25 The mixture was filtered and the methanol slurry was  
repeated one more time. Vacuum drying afforded 7.34 g,  
20.5% of the title polymer.

10. Preparation of  
Poly(trimethylammoniummethacrylatechloride)  
30 co-poly(styrene) (TMAEAC co-Sty)

---

To a 1000 mL, three-necked, round-bottomed flask  
was added the following:  
trimethylammoniummethacrylatechloride (TMAEAC) (99.4 mL  
of a 50% aqueous solution, 53.0 g), divinyl benzene  
35 crosslinking co-monomer (7.00 g), styrene co-monomer  
(40.0 g), and 2-propanol (800 mL). The resulting  
solution was clear. Next, the reaction mixture was

- 19 -

heated to 65°C while being degassed with nitrogen. When the solution had reached 65°C, the catalyst, AIBN (1.50g), was added. The solution immediately began to turn cloudy, indicating that polymerization was proceeding. The reaction was maintained at 65°C for 6 hours, then cooled to 60°C and stirred for an additional 18 hours. It was then allowed to cool to room temperature.

The resulting polymer was filtered and washed on the funnel with isopropanol, and then immediately slurried in 1000 mL of distilled water. The mixture was stirred for 1/2 hour and then 800 mL of methanol was added and the mixture was stirred for an additional 1/2 hour. The mixture was allowed to settle and the supernatant liquid was decanted, leaving a residue of about 750 mL. The residue was then slurried with an additional 750 mL of methanol and stirred for 1/2 hour. The methanol slurry and decantation process was repeated two more times with 800 mL of methanol each time. Next, 800 mL of isopropanol was added and the mixture was stirred for 1/2 hour and then filtered. Finally, 600 mL of isopropanol was added and the mixture was stirred for 1/2 hour. Filtration and vacuum drying afforded 49.2 g, 49.2% of the title polymer.

TMAEAC co-Sty (31%) crosslinked with 8% divinylbenzene crosslinking co-monomer and TMAEAC co-Sty (46%) crosslinked with 6% divinylbenzene crosslinking co-monomer were prepared in analogous fashion by varying the ratio of starting monomers.

11. Preparation of  
Poly(trimethylammoniummethacrylate-chloride co-poly(styrene) (TMAEMC co-Sty)

To a 1000 mL, three-necked, round-bottomed flask was added the following:

trimethylammoniummethacrylatechloride (TMAEMAC)

- 20 -

(38.8 mL of a 50% aqueous solution, 21.7 g), divinyl benzene crosslinking co-monomer (3.72 g), styrene co-monomer (15.66 g), and 2-propanol (2500 mL). The resulting solution was clear. Next, the reaction mixture was heated to 65°C while being degassed with nitrogen. When the solution had reached 65°C, the catalyst, AIBN (0.50g), was added. The solution immediately began to turn cloudy, indicating that polymerization was proceeding. After two hours, the mixture became very thick and an additional 100 mL of isopropanol was added. After five hours the mixture was again very thick so an additional 100 mL of isopropanol was added. The reaction was maintained at 65°C for 6 hours, and then allowed to cool to room temperature.

The resulting polymer was filtered and washed on the funnel with isopropanol, and then immediately slurried in 1000 mL of distilled water. The mixture was stirred for 1/2 hour and then transferred to a blender and blended for five minutes. The polymer slurry was filtered and 1000 mL of distilled water was added and the mixture was stirred for 1/2 hour. The mixture was filtered and the filter cake was slurried two times in 500 mL of methanol each time. Filtration and vacuum drying afforded 30.2 g, 75.9% of the title polymer.

TMAEMC co-Sty (58%) crosslinked with 4% divinylbenzene crosslinking co-monomer, TMAEMC co-Sty (33%) crosslinked with 4% divinylbenzene crosslinking co-monomer, and TMAEMC co-Sty (24%) crosslinked with 4% divinylbenzene crosslinking co-monomer were prepared in analogous fashion by varying the ratio of starting monomers.

12. Preparation of Poly(methacrylamidopropyl-3-(trimethylammonium chloride, co-poly 2, 3, 4, 5, 6-pentafluorostyrene (MAPTAC co-StyF<sub>5</sub>))

To a 1000 mL, three-necked flask, round-bottomed flask was added the following: (methacrylamidopropyl-3-

- 21 -

(trimethylammonium) chloride (MAPTAC) (24.5 mL of a 50% aqueous solution, 13.00 g), divinylbenzene crosslinking co-monomer (1.00 g), pentafluorostyrene (6.00 g), 2-propanol (150 mL), and AIBN (0.50g). The resulting  
5 solution was clear. Next, the reaction mixture was heated to 65°C while being degassed with nitrogen. After a short period of time, the solution began to turn cloudy, indicating that polymerization was proceeding. After five hours the mixture was very thick so an  
10 additional 100 mL of isopropanol was added. The reaction was maintained at 65°C for 24 hours, and then allowed to cool to room temperature.

The resulting polymer was filtered and washed on the funnel with isopropanol and immediately slurried in  
15 500 mL of distilled water. The mixture was stirred for 1/2 hour. The polymer slurry was filtered and 500 mL of distilled water was added and the mixture was stirred for 1/2 hour. The mixture was filtered and the filter cake was slurried two times in 300 mL of methanol each time.  
20 Filtration and air drying afforded 7.74 g of the title co-polymer.

MAPTAC co-StyF<sub>5</sub> (20%) crosslinked with 5% divinylbenzene crosslinking co-monomer, MAPTAC co-StyF<sub>5</sub> (40%) crosslinked with 5% divinylbenzene crosslinking co-  
25 monomer, and MAPTAC co-StyF<sub>5</sub> (45%) crosslinked with 5% divinylbenzene crosslinking co-monomer were prepared in analogous fashion by varying the ratio of starting monomers.

30 13. Preparation of poly(methacrylamidopropyl-3-(trimethylammonium) chloride, co-poly 2-(trimethylammonium) ethyl methacrylate chloride, co-styrene (MAPTAC co-TMAEMC co-Sty)

To a 1000 mL, three-necked flask, round-bottomed  
35 flask was added the following: (methacrylamidopropyl-3-(trimethylammonium) chloride (MAPTAC) (10.40 g of a 50%

- 22 -

aqueous solution, 5.20 g), 2-(trimethylammonium) ethyl methacrylate chloride (TMAEMC) (4.86 g of a 70% aqueous solution, 3.40 g) divinylbenzene crosslinking co-monomer (1.00 g), styrene (10.40 g), 2-propanol (150 mL), and  
5 AIBN (0.50 g). The resulting solution was clear. Next, the reaction mixture was heated to 70°C while being degassed with nitrogen. After a short period of time, the solution began to turn cloudy, indicating that polymerization was proceeding. The reaction was  
10 maintained at 70°C for 24 hours, and then allowed to cool to room temperature.

The resulting polymer was filtered and washed on the funnel with isopropanol and immediately slurried in 500 mL of methanol. The mixture was stirred for 1/2  
15 hour. The polymer slurry was filtered and 400 mL of distilled water was added and the mixture was stirred for 1/2 hour. The mixture was filtered and the water slurry was repeated. The mixture was filtered and the filter cake was slurried two times in 400 mL of methanol each  
20 time. Filtration and air drying afforded 5.39 g of the title co-polymer.

MAPTAC co-TMAEMC (34%) co-Sty (36%) crosslinked with 5% divinylbenzene crosslinking co-monomer, MAPTAC co-TMAEMC (31%) co-Sty (41%) crosslinked with 5%  
25 divinylbenzene crosslinking co-monomer, MAPTAC co-TMAEMC (28%) co-Sty (46%) crosslinked with 5% divinylbenzene crosslinking co-monomer, MAPTAC co-TMAEMC (23%) co-Sty (48%) crosslinked with 5% divinylbenzene crosslinking co-monomer, MAPTAC co-TMAEMC (26%) co-Sty (52%) crosslinked  
30 with 4% divinylbenzene crosslinking co-monomer, MAPTAC co-TMAEMC (17%) co-Sty (53%) crosslinked with 4% divinylbenzene crosslinking co-monomer, MAPTAC co-TMAEMC (15%) co-Sty (55%) crosslinked with 4% divinylbenzene crosslinking co-monomer, and MAPTAC co-TMAEMC (13%) co-  
35 Sty (61.5%) crosslinked with 4% divinylbenzene

- 23 -

crosslinking co-monomer were prepared in analogous fashion by varying the ratio of starting monomers.

5           14. Preparation of  
Poly(trimethylammoniummethacrylate-  
chloride co-poly(isopropylacrylamide)  
(TMAEMAC co-IPA)

The co-monomer isopropylacrylamide (IPA) was first prepared as follows.

10           Acryloyl chloride (63 mL, 70.2 g, 0.775 mol) was dissolved in tetrahydrofuran (200 mL) in a 1 L flask and placed in an ice bath. A solution containing isopropylamine (127.7 mL, 88.67 g, 1.50 mol) was added dropwise, maintaining the temperature at 5-15°C. After addition the solution was stirred for 10 min and the  
15 solid isopropylamine hydrochloride was filtered off and discarded. The solvent was removed in vacuo from the mother liquor and the resulting almost colorless oil, which solidified on standing, was used without further purification to prepare the title co-polymer as follows.

20           To a 1000 mL, three-necked flask, round-bottomed flask was added the following:  
trimethylammoniummethacrylate chloride (76.5 mL of a 50% aqueous solution, 41.18 g, 0.213 mol), methylene bis acrylamide crosslinking co-monomer (2.40 g), IPA co-  
25 monomer (4.52 g, 0.070 mol), and water (200 mL). The resulting solution was clear. The reaction mixture was stirred while being degassed with nitrogen. When the solution had been degassed, the catalyst, consisting of potassium persulfate (0.3 g) and potassium metabisulfate  
30 (0.3 g) was added. The polymerization initiated after 2 minutes and gelled after 3 minutes.

The next morning the gel was transferred to a blender and 1000 mL of water was added. After blending for a few seconds, the polymer had swelled to take up all  
35 of the water. The swollen polymer was blended in several portions with isopropanol several times to dehydrate it.

- 24 -

The resulting polymer was filtered and washed on the funnel with isopropanol and vacuum dried to afford 36.8 g of the title co-polymer.

5        15. Preparation of  
Poly(methacrylamidopropyltrimethylammonium-  
chloride) co-poly(vinyl pyridine) (MAPTAC co-VP)

To a 1000 mL, three-necked flask, round-bottomed flask was added the following: (methacrylamidopropyl-3-  
10 (trimethylammonium) chloride (MAPTAC) (40 mL of a 50% aqueous solution, 21.0 g), divinyl benzene crosslinking co-monomer (2.25 g), vinyl pyridine (14.0 g, 0.133 mol), conc. hydrochloric acid (11 mL, 0.133 mol), 2-propanol (300 mL), and AIBN (0.67 g). The resulting solution was  
15 clear. Next, the reaction mixture was heated to 60°C while being degassed with nitrogen. After a short period of time, the solution began to turn cloudy, indicating that polymerization was proceeding. The reaction was maintained at 60°C for 20 hours, and then allowed to cool  
20 to room temperature.

The resulting polymer was filtered and washed on the funnel with isopropanol and immediately slurried in 1000 mL of distilled water. The mixture was stirred for 1 hour. The polymer slurry was filtered, washed on the  
25 funnel with methanol, and then slurried in 600 mL of methanol for one hour. Filtration and air drying afforded 20.4 g of co-polymer.

30        16. Preparation of  
Poly(trimethylammoniummethylemethacrylate-  
chloride co-poly(p-fluorostyrene)  
(TMAEMC co-F<sub>1</sub>Sty)

To a 500 mL flask was added the following:  
trimethylammoniummethylemethacrylate chloride (TMAEMC)  
(11.0 g of a 70% aqueous solution, 7.70 g),  
35 divinylbenzene crosslinking co-monomer (0.50 g), p-fluorostyrene co-monomer (4.00 g), 2-propanol (125 mL) and AIBN (0.25 g). The resulting solution was clear.

- 25 -

Next, the reaction mixture was heated to 65°C while being degassed with nitrogen. The solution immediately began to turn cloudy, indicating that polymerization was proceeding. The reaction was maintained at 65°C for 6 hours, and then allowed to cool to room temperature.

The solvent was removed by decantation and the polymer was immediately slurried in 250 mL of distilled water. The mixture was stirred for 1/2 hour and then decanted. The water slurry was repeated three more times. Finally, the polymer was slurried in 400 mL of methanol. Filtration and vacuum drying afforded 5.42 g, 44.4% of the title co-polymer.

TMAEMC co-F<sub>1</sub>Sty (24%) crosslinked with 4% divinylbenzene crosslinking co-monomer was prepared in analogous fashion by varying the ratio of the starting monomers.

17. Preparation of  
Poly(methacrylamidopropyltrimethyl ammonium  
chloride) co-poly(hexafluorobutyl  
methacrylate) (MAPTAC coF<sub>6</sub>BMA)

To a 1000 mL, three-necked flask, round-bottomed flask was added the following: (methacrylamidopropyl-3-(trimethylammonium) chloride (MAPTAC) (28.5 mL of a 50% aqueous solution, 15.0 g), divinylbenzene crosslinking co-monomer (1.00 g), hexafluorobutyl methacrylate (4.00 g), 2-propanol (150 mL), and AIBN (0.50 g). The resulting solution was clear. Next, the reaction mixture was heated to 60°C while being degassed with nitrogen. After a short period of time, the solution began to turn cloudy, indicating that polymerization was proceeding. The reaction was maintained at 60°C for 24 hours, and then allowed to cool to room temperature.

The resulting polymer was filtered and washed on the funnel with isopropanol and immediately slurried in 500 mL of distilled water. The mixture was stirred for 1 hour. The polymer slurry was filtered and the water

- 26 -

slurry was repeated one more time. The polymer was then slurried in 500 mL of methanol for one hour and filtered. The methanol slurry was repeated one more time. Finally the polymer was slurried in 400 mL of isopropanol and stirred overnight. Filtration and air drying afforded 7.52 g of the title co-polymer.

18. Preparation of  
Poly(trimethylammoniummethacrylate chloride)  
co-poly(hexafluoroisopropyl acrylate)  
(TMAEAC co-F<sub>6</sub>IA)

To a 1000 mL, three-necked flask, round-bottomed flask was added the following:  
trimethylammoniummethacrylate chloride (30.0 mL of a 50% aqueous solution, 15.0 g), divinylbenzene crosslinking co-monomer (1.00 g), F<sub>6</sub>IPA co-monomer (4.00 g), AIBN (0.50 g), and isopropanol (150 mL). The resulting solution was clear. The reaction mixture was stirred while being degassed with nitrogen and heated to 60°C. After 18 hours the reaction mixture was allowed to cool to room temperature and the solvent was removed by decanting. The residual polymer was slurried in 400 mL of water, stirred for one hour and filtered. The water slurry was repeated one more time. Next the polymer was slurried two times in methanol. Finally, the polymer was slurried in 200 mL of isopropanol, stirred for two hours and filtered. Air drying afforded 5.59 g of the title polymer.

19. Preparation of  
Poly(methacrylamidopropyltrimethyl ammonium chloride) co-poly(heptadecafluorodecyl methacrylate) (MAPTAC coF<sub>17</sub>DecMA)

To a 1000 mL, three-necked flask, round-bottomed flask was added the following: (methacrylamidopropyl-3-(trimethylammonium) chloride (MAPTAC) (28.5 mL of a 50% aqueous solution, 15.0 g), divinylbenzene crosslinking co-monomer (1.00 g), heptadecafluorodecyl methacrylate

- 27 -

(4.00 g), 2-propanol (150 mL), and AIBN (0.40 g). The resulting solution was clear. Next, the reaction mixture was heated to 65°C while being degassed with nitrogen. After a short period of time, the solution began to turn  
5 cloudy, indicating that polymerization was proceeding. After four hours, the reaction mixture had gotten very thick and 100 mL more isopropanol was added. The reaction was maintained at 65°C for 18 hours, and then allowed to cool to room temperature.

10 The resulting polymer was filtered and washed on the funnel with isopropanol and immediately slurried in 600 mL of distilled water. The mixture was stirred for 1 hour. The polymer slurry was filtered and the water slurry was repeated one more time. The polymer was then  
15 slurried in 500 mL of methanol for one hour and filtered. Air drying afforded 17.73 g of co-polymer.

20. Preparation of poly(methacrylamidopropyl-3-(trimethylammonium) chloride, co-poly 2-(trimethylammonium) ethyl acrylate chloride,  
20 co-poly styrene (MAPTAC co-TMAEAC co-Sty)

To a 1000 mL, three-necked flask, round-bottomed flask was added the following: (methacrylamidopropyl-3-(trimethylammonium) chloride (MAPTAC) (10.00 g of a 50% aqueous solution, 5.00 g), 2-(trimethylammonium) ethyl  
25 methacrylate chloride (TMAEAC) ( 6.00 g of a 50% aqueous solution, 3.00 g) divinylbenzene crosslinking co-monomer (1.00 g), styrene (11.00 g), 2-propanol (150 mL), and AIBN (0.25g). The resulting solution was clear. Next, the reaction mixture was heated to 70°C while being  
30 degassed with nitrogen. After a short period of time, the solution began to turn cloudy, indicating that polymerization was proceeding. The reaction was maintained at 70°C for 24 hours, and then allowed to cool to room temperature.

35 The resulting polymer was filtered and washed on the funnel with isopropanol and immediately slurried in 500

- 28 -

mL of methanol. The mixture was stirred for 1/2 hour . The polymer slurry was allowed to settle and decanted. 200 mL of distilled water was added and the mixture was stirred for 1/2 hour. The mixture was decanted and the  
5 water slurry was repeated with 400 mL. The mixture was decanted and the polymer was slurried two times in 200 mL of methanol each time. Filtration and air drying afforded 2.76 g of the title co-polymer.

MAPTAC co-TMAEAC (10%) co-Sty (60%) crosslinked with  
10 5% divinylbenzene crosslinking co-monomer was prepared in analogous fashion by varying the ratio of starting monomers.

21. Preparation of poly-2-(trimethylammonium) ethyl  
15 acrylate chloride co-poly 2,3,4,5,6-  
pentafluorostyrene (TMAEAC co-StyF<sub>5</sub>)

To a 1000 mL, three-necked flask, round-bottomed flask was added the following: 2-(trimethylammonium) ethyl acrylate chloride (TMAEAC) (24.0 mL of a 50% aqueous solution, 13.00 g), divinylbenzene crosslinking  
20 co-monomer (1.00 g), pentafluorostyrene (6.00 g), 2-propanol (150 mL), and AIBN (0.50g). The resulting solution was clear. Next, the reaction mixture was heated to 65°C while being degassed with nitrogen. After a short period of time, the solution began to turn  
25 cloudy, indicating that polymerization was proceeding. After two hours the mixture was very thick so an additional 100 mL of isopropanol was added. The reaction was maintained at 65°C for 22 hours, and then allowed to cool to room temperature.

30 The resulting polymer was filtered and washed on the funnel with isopropanol and immediately slurried in 400 mL of distilled water. The mixture was stirred for 1/2 hour. The polymer slurry was filtered and 600 mL of distilled water was added and the mixture was stirred for  
35 1/2 hour. The mixture was filtered and the filter cake

- 29 -

was slurried in 400 mL of methanol. Filtration and air drying afforded 7.26 g of the title co-polymer.

TMAEAC co-StyF<sub>5</sub> (20%) crosslinked with 5% divinylbenzene crosslinking co-monomer was prepared in 5 analogous fashion by varying the ratio of starting monomers.

22. Preparation of 2-(trimethylammonium) ethyl methacrylate chloride, co-poly 2,3,4,5,6-pentafluorostyrene (TMAEMC co-StyF<sub>5</sub>)

10 To a 1000 mL, three-necked flask, round-bottomed flask was added the following: 2-(trimethylammonium) ethyl methacrylate chloride (TMAEMC) (19.52 of a 70% aqueous solution, 13.66 g), divinylbenzene crosslinking co-monomer (1.00 g), pentafluorostyrene (9.18 g), 2-  
15 propanol (150 mL), and AIBN (0.40g). The resulting solution was clear. Next, the reaction mixture was heated to 70°C while being degassed with nitrogen. After a short period of time, the solution began to turn cloudy, indicating that polymerization was proceeding.  
20 After 1.5 hours the mixture was very thick so an additional 50 mL of isopropanol was added. The reaction was maintained at 70°C for 5 hours, and then allowed to cool to room temperature.

The resulting polymer was filtered and washed on the  
25 funnel with isopropanol and immediately slurried in 500 mL of distilled water. The mixture was stirred for 1/4 hour. The polymer slurry was filtered and 500 mL of distilled water was added and the mixture was stirred for 1/4 hour. The water slurry was repeated one more time.  
30 The mixture was filtered and the filter cake was slurried three times in 300 mL of methanol each time. Filtration and air drying afforded 1.26 g of the title co-polymer.

TMAEMC co-StyF<sub>5</sub> (24%) crosslinked with 4% divinylbenzene crosslinking co-monomer and TMAEMC co-  
35 StyF<sub>5</sub> (39%) crosslinked with 4% divinylbenzene

- 30 -

crosslinking co-monomer were prepared in analogous fashion by varying the ratio of starting monomers.

23. Preparation of Poly(ethyleneimine)

Polyethyleneimine (120 g of a 50% aqueous solution; Scientific Polymer Products) was dissolved in water (250 mL). Epichlorohydrin (22.1 mL) was added dropwise. The solution was heated to 60°C for 4 h, after which it had gelled. The gel was removed, blended with water (1.5 L) and the solid was filtered off, rinsed three times with water (3 L) and twice with isopropanol (3 L), and the resulting gel was dried in a vacuum oven to yield 81.2 g of the title polymer.

24. Preparation of Poly(methacrylamidopropyl-3-(trimethylammonium) chloride, co-poly(2-(trimethylammonium) ethylmethacrylate chloride) co-poly 2,3,4,5,6-pentafluorostyrene (MAPTAC co-TMAEMC co-StyF<sub>5</sub>)

To a 1000 mL, three-necked flask, round-bottomed flask was added the following: (methacrylamidopropyl-3-(trimethylammonium) chloride (MAPTAC) (10.00 of a 50% aqueous solution, 5.00 g), 2-(trimethylammonium) ethyl methacrylate chloride (TMAEMC) (5.71 g of a 70% aqueous solution, 4.00 g), divinylbenzene crosslinking co-monomer (1.00 g), pentafluorostyrene (10.00 g), 2-propanol (150 mL), and AIBN (0.50g). The resulting solution was clear. Next, the reaction mixture was heated to 70°C while being degassed with nitrogen. After a short period of time, the solution began to turn cloudy, indicating that polymerization was proceeding. The reaction was maintained at 70°C for 24 hours, and then allowed to cool to room temperature.

The resulting polymer was filtered and washed on the funnel with isopropanol and immediately slurried in 500 mL of methanol. The mixture was stirred for 1/4 hour. The polymer slurry was filtered and then slurried 3 times in 300 mL of water each time. The last time the polymer

- 31 -

slurry was blended for 5 minutes. The mixture was filtered and the filter cake was slurried two times in 300 mL of methanol each time. Filtration and vacuum drying afforded 9.74 g of co-polymer.

- 5        25. Preparation of Poly(trimethylammonium) ethyl acrylate chloride, co-poly(2-(trimethylammonium) ethylmethacrylate chloride) co-styrene (TMAEAC, co-TMAEMC, co-Sty)

To a 1000 mL, three-necked flask, round-bottomed  
10 flask was added the following: 2-(trimethylammonium) ethyl acrylate chloride (TMAEAC) (6.00 g of a 50% aqueous solution, 3.00 g), 2-(trimethylammonium) ethyl methacrylate chloride (TMAEMC) (4.29 g of a 70% aqueous solution, 3.00 g), divinylbenzene crosslinking co-monomer  
15 (1.00 g), styrene (13.00 g), 2-propanol (150 mL), and AIBN (0.50g). The resulting solution was clear. Next, the reaction mixture was heated to 70°C while being degassed with nitrogen. After a short period of time, the solution began to turn cloudy, indicating that  
20 polymerization was proceeding. The reaction was maintained at 70°C for 24 hours, and then allowed to cool to room temperature.

The resulting polymer was decanted and immediately slurried in 500 mL of methanol. The mixture was stirred  
25 for 1/2 hour. The polymer slurry was filtered and 500 mL of distilled water was added. The mixture was then stirred for 1/2 hour and blended for 10 minutes. The mixture was allowed to settle and the water was decanted. The water slurry was repeated two more times and the  
30 decantation residue was slurried two times in 400 mL of methanol each time, settling and decanting each time. Vacuum drying afforded 8.03 g of the title co-polymer.

- 32 -

26. Preparation of Poly(methacrylamidopropyl-3-(trimethylammonium) chloride, co-poly(2-(trimethylammonium) ethylacrylate chloride) co-poly 2,3,4,5,6-pentafluorostyrene (MAPTAC co-TMAEAC co-StyF<sub>5</sub>)

To a 1000 mL, three-necked flask, round-bottomed flask was added the following: (methacrylamidopropyl-3-(trimethylammonium) chloride (MAPTAC) (8.00 g of a 50% aqueous solution, 4.00 g), 2-(trimethylammonium) ethyl acrylate chloride (TMAEMA) (6.00 g of a 50% aqueous solution, 3.00 g), divinylbenzene crosslinking co-monomer (1.00 g), pentafluorostyrene (12.00 g), 2-propanol (150 mL), and AIBN (0.50g). The resulting solution was clear. Next, the reaction mixture was heated to 70°C while being degassed with nitrogen. After a short period of time, the solution began to turn cloudy, indicating that polymerization was proceeding. The reaction was maintained at 70°C for 24 hours, and then allowed to cool to room temperature.

The resulting polymer was filtered and washed on the funnel with isopropanol and immediately slurried in 400 mL of methanol. The mixture was stirred for 1/2 hour. The polymer slurry was filtered and then slurried 2 times in 250 mL of water each time. The last time the polymer slurry was blended for 5 minutes. The mixture was filtered and the filter cake was slurried two times in 250 mL of methanol each time. Filtration and vacuum drying afforded 7.80 g of co-polymer.

27. Preparation of Poly(trimethylammonium) ethyl acrylate chloride, co-poly(2-(trimethylammonium) ethylmethacrylate chloride) co-2,3,4,5,6-pentafluorostyrene (TMAEAC, co-TMAEMC, co-StyF<sub>5</sub>)

To a 1000 mL, three-necked flask, round-bottomed flask was added the following: 2-(trimethylammonium) ethyl acrylate chloride (TMAEAC) (6.00 g of a 50% aqueous solution, 3.00 g), 2-(trimethylammonium) ethyl

- 33 -

methacrylate chloride (TMAEMC) (4.29 g of a 70% aqueous solution, 3.00 g), divinylbenzene crosslinking co-monomer (1.00 g), pentafluorostyrene (13.00 g), 2-propanol (150 mL), and AIBN (0.50g). The resulting solution was clear.

- 5 Next, the reaction mixture was heated to 70°C while being degassed with nitrogen. After a short period of time, the solution began to turn cloudy, indicating that polymerization was proceeding. The reaction was maintained at 70°C for 24 hours, and then allowed to cool  
10 to room temperature.

- The resulting polymer was decanted and immediately slurried in 400 mL of methanol. The mixture was stirred for 1/2 hour. The polymer slurry was filtered and then slurried two times in 200 mL of water each time. The  
15 second time the polymer slurry was blended for 5 minutes. The mixture was filtered and the filter cake was slurried two times in 200 mL of methanol each time. Vacuum drying afforded 6.87 g of co-polymer.

#### Testing of Polymers

##### 20 A. Preparation of Artificial Intestinal Fluid

- Sodium carbonate (1.27g) and sodium chloride (1.87g) were dissolved in 400 mL of distilled water. To this solution was added a mixture of purified bile acids, consisting of taurocholic acid (0.138g, 0.24mmol),  
25 glycocholic acid (0.292g, 0.60mmol), glycodeoxycholic acid (0.085mmol, 0.18mmol) and glycochenodeoxycholic acid (0.085mmol, 0.18mmol). The pH of the solution was adjusted to 7.20 with acetic acid. This solution was used for the testing of the various polymers. The total  
30 bile salt concentration in this solution is 3 millimolar, a concentration approximately equal to that found in normal physiological solutions in the duodenum.

Polymers were tested as follows.

- To a 40mL centrifuge tube was added 0.25g of polymer  
35 and 20 mL of the artificial small fluid prepared as

- 34 -

described above. The mixture was stirred in a water bath maintained at 37°C for three hours. The mixture was then centrifuged and the supernatant liquid, being slightly cloudy, was filtered. The filtrate was analyzed for total 3-hydroxy steroid content by an enzymatic assay using 3 $\alpha$ -hydroxy steroid dehydrogenase, as described below.

#### Enzymatic Assay for Total Bile Salt Content

Four stock solutions were prepared.

10 Solution 1. Tris-HCl buffer, containing 0.133M Tris, 0.666mM EDTA at pH 9.5.

Solution 2. Hydrazine hydrate solution, containing 1M hydrazine hydrate at pH 9.5.

15 Solution 3. NAD<sup>+</sup> solution, containing 7mM NAD<sup>+</sup> at pH 7.0.

Solution 4. HSD solution, containing 2units/mL in Tris-HCl buffer (0.03M Tris, 1mM EDTA) at pH 7.2.

To a 3 mL cuvette was added 1.5 mL of Solution 1, 1.0 mL of Solution 2, 0.3 mL of Solution 3, 0.1 mL of Solution 4 and 0.1 mL of supernatant/filtrate from a polymer test as described above. The solution was placed in a UV-VIS spectrophotometer and the absorbance (O.D.) of NADH at 340 nm was measured. The bile salt concentration was determined from a calibration curve prepared from dilutions of the artificial intestinal fluid prepared as described above.

25 All of the polymers previously described were tested in the above manner and all were efficacious in removing bile salts from the artificial intestinal fluid.

#### 30 Use

The polymers according to the invention may be administered orally to a patient in a dosage of about 1 mg/kg/day to about 10 g/kg/day; the particular dosage will depend on the individual patient (e.g., the patient's weight and the extent of bile salt removal

- 35 -

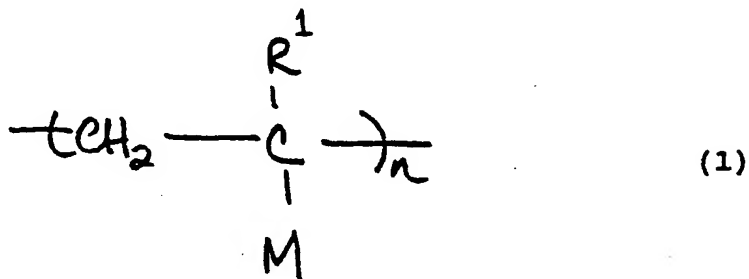
required). The polymer may be administered either in hydrated or dehydrated form, and may be flavored if necessary to enhance patient acceptability; additional ingredients such as artificial coloring agents may be  
5 added as well.

Examples of suitable forms for administration include pills, tablets, capsules, and powders (for sprinkling on food). The pill, tablet, capsule, or powder can be coated with a substance capable of  
10 protecting the composition from the gastric acid in the patient's stomach for a period of time sufficient to allow the composition to pass undisintegrated into the patient's small intestine. The polymer may be administered alone or in combination with a  
15 pharmaceutically acceptable carrier substance, e.g., magnesium carbonate, lactose, or a phospholipid with which the polymer can form a micelle.

Other embodiments are within the following claims.  
What is claimed is:

- 36 -

1. A method for removing bile salts from a patient by ion exchange comprising administering to said patient a therapeutically effective amount of one or more highly crosslinked polymers characterized by a repeat unit  
5 having the formula



or copolymer thereof, where n is an integer; R<sup>1</sup> is H or a

- C<sub>1</sub>-C<sub>8</sub> alkyl group; M is  $\text{-}\overset{\text{O}}{\parallel}\text{C-Z-R}^2$  or  $\text{-Z-R}^2$ ; Z is O, NR<sup>3</sup>, S,  
10 or (CH<sub>2</sub>)<sub>m</sub>; m = 0-10; R<sup>3</sup> is H or a C<sub>1</sub>-C<sub>8</sub> alkyl group; and R<sup>2</sup> is



where p = 0-10, and each R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup>, independently, is H, a C<sub>1</sub>-C<sub>8</sub> alkyl group, or an aryl group,

- 15 said polymers being non-toxic and stable once ingested.

2. The method of claim 1 wherein said polymer is crosslinked by means of a multifunctional crosslinking co-monomer, said co-monomer being present in an amount  
20 from about 1-25% by weight, based upon total monomer weight.

- 37 -

3. The method of claim 2 wherein said crosslinking co-monomer is present in an amount from about 2.5-20% by weight, based upon total monomer weight.

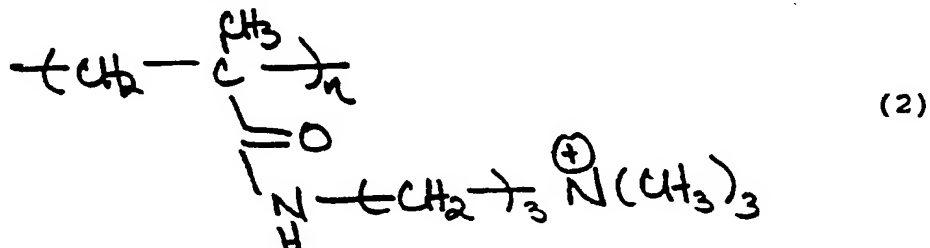
4. The method of claim 1 wherein said polymer  
5 further comprises one or more hydrophobic co-monomers.

5. The method of claim 4 wherein said hydrophobic co-monomer comprises styrene and fluorinated derivatives thereof; vinyl naphthalene and fluorinated derivatives thereof; ethyl vinylbenzene and fluorinated derivatives  
10 thereof; N-alkyl and N-aryl derivatives of acrylamide and methacrylamide, and fluorinated derivatives thereof; alkyl and aryl acrylates and fluorinated derivatives thereof; and alkyl and aryl methacrylates and fluorinated derivatives thereof.

15 6. The method of claim 1 wherein said polymer further comprises one or more positively charged co-monomers.

7. The method of claim 6 wherein said positively charged co-monomer comprises vinyl pyridine,  
20 dimethylaminomethyl styrene, and vinyl imidazole.

8. The method of claim 1 wherein said polymer is characterized by a repeat unit having the formula



or copolymer thereof.

- 38 -

9. The method of claim 8 wherein said polymer further comprises, as a co-monomer, n-butylmethacrylamide.

10. The method of claim 8 wherein said polymer further comprises, as a co-monomer, hexafluorobutylmethacrylate.

11. The method of claim 8 wherein said polymer further comprises, as a co-monomer, heptadecafluorodecylmethacrylate.

10 12. The method of claim 8 wherein said polymer further comprises, as a co-monomer, styrene or a fluorinated derivative thereof.

13. The method of claim 8 wherein said polymer further comprises, as a co-monomer, 2-vinyl naphthalene.

15 14. The method of claim 8 wherein said polymer further comprises, as a co-monomer, 4-vinyl imidazole.

15. The method of claim 8 wherein said polymer further comprises, as a co-monomer, vinyl pyridine.

16. The method of claim 8 wherein said polymer further comprises, as a co-monomer, trimethylammoniummethacrylate.

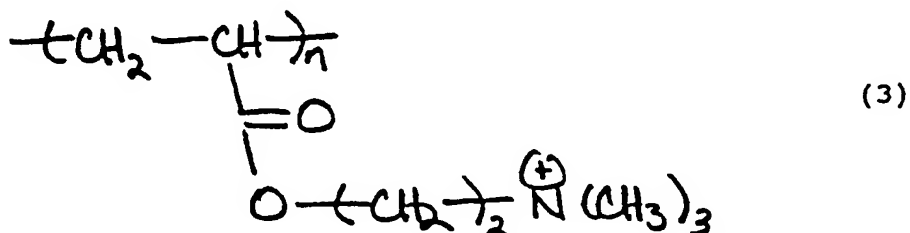
17. The method of claim 16 wherein said polymer further comprises, as a co-monomer, styrene or a fluorinated derivative thereof.

- 39 -

18. The method of claim 8 wherein said polymer further comprises, as a co-monomer, trimethylammoniummethyacrylate.

19. The method of claim 18 wherein said polymer further comprises, as a co-monomer, styrene or a fluorinated derivative thereof.

20. The method of claim 1 wherein said polymer is characterized by a repeat unit having the formula



10 or copolymer thereof.

21. The method of claim 20 wherein said polymer further comprises, as a co-monomer, isopropylacrylamide.

22. The method of claim 20 wherein said polymer further comprises, as a co-monomer, styrene or a fluorinated derivative thereof.

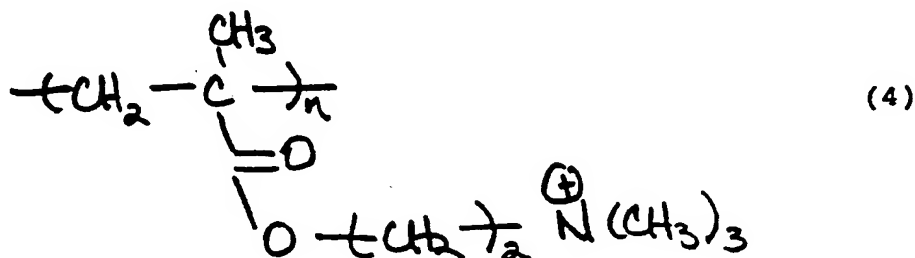
23. The method of claim 20 wherein said polymer further comprises, as a co-monomer hexafluoroisopropylacrylate.

24. The method of claim 20 wherein said polymer further comprises, as a co-monomer, trimethylammoniummethacrylate.

25. The method of claim 24 wherein said polymer further comprises, as a co-monomer, styrene or a fluorinated derivative thereof.

- 40 -

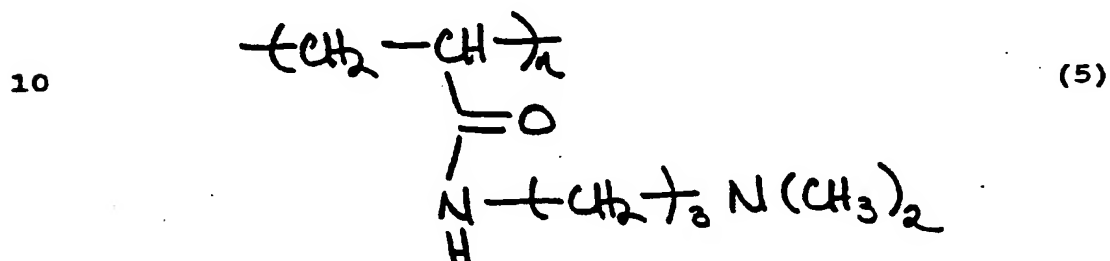
26. The method of claim 1 wherein said polymer is characterized by a repeat unit having the formula



or copolymer thereof.

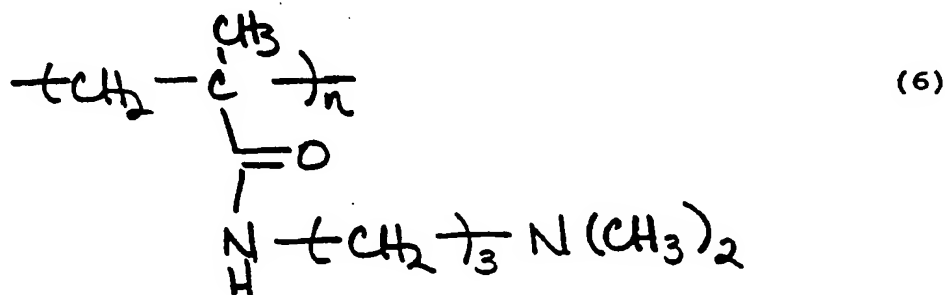
5 27. The method of claim 26 wherein said polymer further comprises, as a co-monomer, styrene or a fluorinated derivative thereof.

28. The method of claim 1 wherein said polymer is characterized by a repeat unit having the formula



or copolymer thereof.

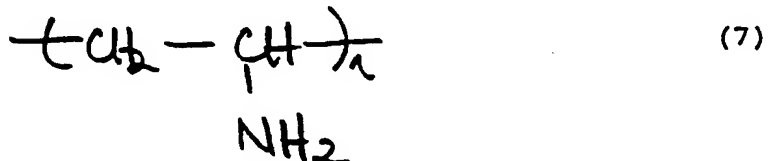
29. The method of claim 1 wherein said polymer is characterized by a repeat unit having the formula



- 41 -

or copolymer thereof.

30. The method of claim 1 wherein said polymer is characterized by a repeat unit having the formula



5 or copolymer thereof.

31. The method of claim 30 wherein said polymer further comprises, as a co-monomer, ethyl vinylbenzene.

32. The method of claim 1 wherein said polymer is characterized by a repeat unit having the formula



or copolymer thereof.

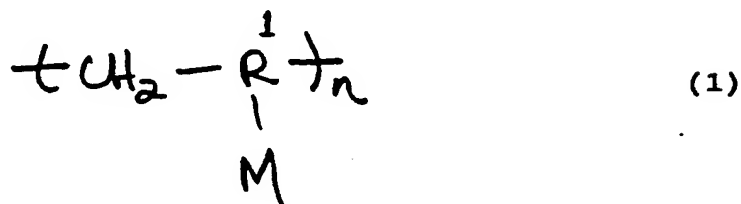
33. The method of claim of 1 wherein said polymer further comprises one or more exchangeable counterions.

34. The method of claim 33 wherein at least one of  
 15 said counterions comprises  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{CH}_3\text{OSO}_3^-$ ,  $\text{HSO}_4^-$ ,  $\text{SO}_4^{2-}$ ,  
 $\text{HCO}_3^-$ ,  $\text{CO}_3^-$ , acetate, lactate, succinate, propionate,  
 butyrate, ascorbate, citrate, maleate, folate, an amino

- 42 -

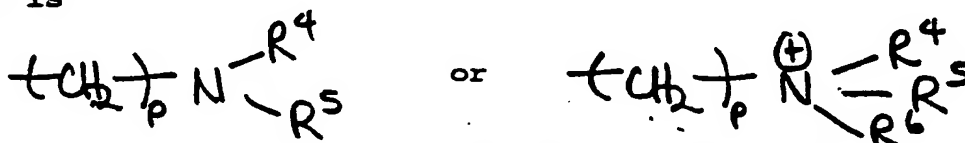
acid derivative, a nucleotide, a lipid, or a phospholipid.

35. A therapeutic composition effective for removing bile salts by ion exchange comprising a therapeutic amount of a highly crosslinked polymer characterized by a repeat unit having the formula



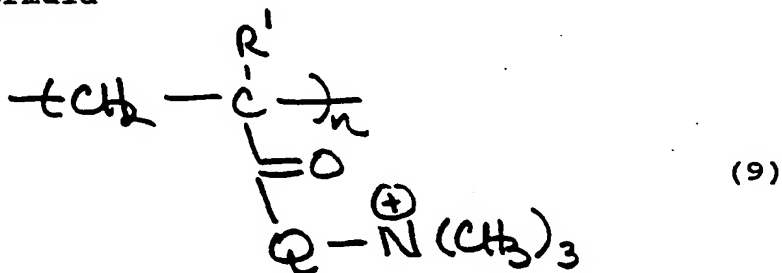
or copolymer thereof, where n is an integer; R<sup>1</sup> is H or a

- 10 C<sub>1</sub>-C<sub>8</sub> alkyl group; M is -C-Z-R<sup>2</sup> or -Z-R<sup>2</sup>; Z is O, NR<sup>3</sup>, S, or (CH<sub>2</sub>)<sub>m</sub>; m = 0-10; R<sup>3</sup> is H or a C<sub>1</sub>-C<sub>8</sub> alkyl group; and R<sup>2</sup> is



- where p = 0-10, and each R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup>, independently, is  
 15 H, a C<sub>1</sub>-C<sub>8</sub> alkyl group, or an aryl group,  
 said polymer being non-toxic and stable once ingested.

36. A highly crosslinked polymer composition comprising a polymer characterized by a repeat unit  
 20 having the formula



- 43 -

where  $R^1$  is H or methyl, Q is  $-NH-(CH_2)_3-$  or  $-O-(CH_2)_2$  and n is an integer, and at least one additional co-monomer selected from the group consisting essentially of vinyl naphthalene, vinylimidazole, fluorinated derivatives of styrene, and fluorinated alkyl methacrylates.

37. The composition of claim 36 wherein  $R^1$  is methyl and Q is  $-NH-(CH_2)_3-$ .

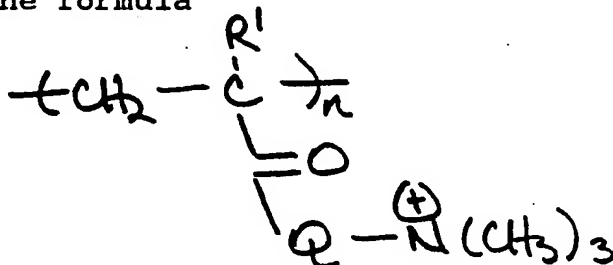
38. The composition of claim 37 further comprising, as a co-monomer, trimethylammoniummethacrylate or trimethylammoniummethylethacrylate.

39. The composition of claim 36 wherein Q is  $-O-(CH_2)_2-$ .

40. The composition of claim 36 wherein said fluorinated derivative of styrene comprises p-fluorostyrene and pentafluorostyrene.

41. The composition of claim 36 wherein said fluorinated alkyl methacrylate comprises hexafluorobutyl methacrylate and heptafluorodecyl methacrylate.

42. A highly crosslinked polymer composition comprising a polymer characterized by a repeat unit having the formula



(10)

where  $R^1$  is H or methyl, Q is  $-NH-(CH_2)_3-$  or  $-O-(CH_2)_2$  and n is an integer, and, as additional co-monomers, (a)

- 44 -

styrene and (b) trimethylammoniummethacrylate or trimethylammoniummethacrylate when  $R^1$  is methyl and Q is  $-\text{NH}-(\text{CH}_2)_3-$ , and methylacrylamidopropyltrimethylammonium when  $R^1$  is H or 5 methyl and Q is  $-\text{O}-(\text{CH}_2)_2-$ .

43. A method of synthesizing a highly crosslinked polymer having hydrophilic and hydrophobic units comprising reacting hydrophilic and hydrophobic monomers in the presence of an alcoholic solvent.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US94/06042

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 31/74, 9/00, 9/48, 9/54, 9/52, 9/20, 9/28, 9/14

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/78.08, 78.01, 78.07, 400, 451, 458, 457, 464, 474, 483, 489, 490

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 4,557,930 (KIHARA et al.) 10 December 1985, see entire document.	1-42
A	US, A, 4,412,011 (KIHARA et al.) 25 October 1983, see entire document.	1-42
X	US, A, 3,953,406 (MARSH, JR.) 27 April 1976, see claim 1.	43

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be part of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

07 JULY 1994

Date of mailing of the international search report

07 SEP 1994

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

RICHARD JONES

Telephone No. (703) 308-2351

Form PCT/ISA/210 (second sheet)(July 1992)\*

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US94/06042

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US94/06042

## A. CLASSIFICATION OF SUBJECT MATTER: US CL :

424/78.08, 78.01, 78.07, 400, 451, 458, 457, 464, 474, 483, 489, 490

## BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

Group I; claims 1-42, methods for removing bile salts and compositions used for removing bile salts which share the common technical feature of a crosslinked polymer characterized by a repeat unit having a specified formula.

Group II; claim 43, a method for making a crosslinked polymer of non specified formula that may or may not have the repeat units of group I.

Group II is not limited to the common technical feature of a crosslinked polymer characterized by a repeat unit having a specified formula as are all claims of Group I.